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ANDROGRAPHOLIDE: THE ACTIVE PRINCIPLE OF *KALMEGH*

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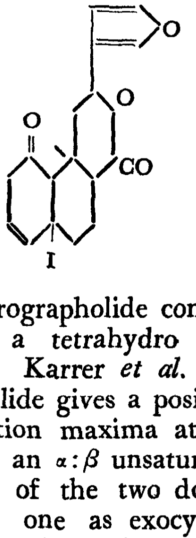
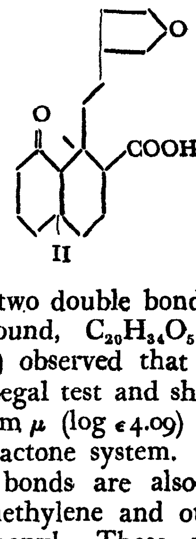
ABSTRACT

The paper gives a critical review of the chemistry of andrographolide, the active principle of *Andrographis paniculata* Nees, the common bitter popularly known as *Kalmegh*. It was isolated in 1911 and although the gross chemical nature as a trihydroxy diterpene lactone was known much earlier, it took more than fifty years for its complete elucidation.

Andrographis paniculata Nees is a shrub belonging to the family Acanthaceae and grows wildly throughout the plains of India. It is well reputed under the name *Kalmegh* as a common bitter prescribed to children in liver troubles (Kirtikar & Basu, 1935) and as such tinctures and extracts of it have earned recognition in the Indian Pharmacopoeia, 1955.

An attempt to isolate the active principle was made as early as in 1911 and a colourless neutral substance was obtained which was called andrographide (Boorsma, 1911). The bitter principle was isolated in a pure crystalline form $C_{20}H_{30}O_5$, m.p. 227.5° by Gorter (1911) who found it to be a lactone. He changed its name from andrographide to andrographolide which is still in use to-day (Gorter, 1911, 1914). It is extremely bitter, sparingly soluble in benzene, ether, acetone, freely soluble in chloroform, methyl alcohol, ethyl alcohol and almost insoluble in water. It forms a triacetate, $C_{20}H_{27}O_2(OAC)_3$, m.p. 129° and the lactone ring opens up with alkali to give andrographolic acid, $C_{20}H_{32}O_6$, m.p. 188°. Gorter & Harye (1914) pointed out that one of the three acetyl groups of triacetylandrographolide is replaced by hydrogen on reduction with aluminium amalgam at ordinary temperature to form diacetyldesoxyandrographolide, $C_{20}H_{28}O_2(OAC)_2$, m.p. 121°. It adds two atoms of bromine giving diacetyldesoxydibromoandrographolide, m.p. 168°. The dibromide can be reduced with hydrogen to a diacetyltetrahydrodesoxyandrographolide, $C_{20}H_{32}O_2(OAC)_2$, m.p. 90-91°. All the five oxygen atoms in andrographolide are thus accounted for, three in hydroxyl functions and two in the lactone system. From the behaviour of one of the acetyl groups in the triacetate towards aluminium amalgam Gorter *et al.* (1914) suggested that this hydroxyl group was tertiary in nature. An identical view was upheld by later workers (Chakravarti & Chakravarti, 1951, 1952; Schwyzer, Biswas and Karrer, 1951). But they overlooked the possibility of influence of a double bond on the supposed hydroxyl group that so easily dehydrates. The experimental condition under which the desoxy compound was produced was in reality the condition of hydrogenolysis. It might be mentioned that the same phenomenon was observed during hydrogenation of deoxycolumbin (I) (Barton & Elad, 1956) where the proximity of a double bond in a furan ring dictates hydrolysis of the lactone bridge followed by dehydration, which subsequently furnishes the octahydro derivative (II).


I


II

Andrographolide contains two double bonds as it forms a tetrahydro compound, $C_{20}H_{34}O_5$, m.p. 186-9°. Karrer *et al.* (1951) observed that andrographolide gives a positive Legal test and shows an absorption maxima at 223 mμ (log ϵ 4.09) indicative of an $\alpha:\beta$ unsaturated lactone system. So the nature of the two double bonds are also determined, one as exocyclic methylene and other as conjugated to lactone carbonyl. These workers could show that while the former is hydrogenated under mild conditions by saturation with hydrogen gas the latter requires a catalyst for its hydrogenation.

Kondo, Kobayashi & Ono (1950) however observed that during hydrogenation of andrographolide in glacial acetic acid in presence of palladised charcoal four moles of hydrogen is absorbed to form a syrupy octahydro derivative, m.p. 140-5°. This gives a triacetyl derivative m.p. 188-98°. Deacetylation of octahydrotriacetylandrographolide gives two isomeric octahydroanhydroandrographolide, (m.p. 140-5° and m.p. 198°) together with some impure crystals m.p. 190-98° given a diacetate, m.p. 96°. They concluded that andrographolide is a monocyclic diterpene having four double bonds, a lactone linkage and three tertiary hydroxyl groups.

Karrer *et al.* (1951) observed that diacetyl anhydroandrographolide, m.p. 162-63° prepared by addition of acetic anhydride to a solution of andrographolide in pyridine, has three double bonds instead of original two in andrographolide. It gives a positive Legal test and shows absorption maxima at 223 mμ and 299 mμ, the latter being due to three double bonds in conjugation.

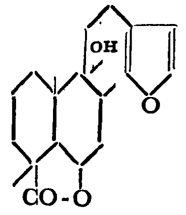
A critical survey of the findings stated above will show that andrographolide contains one isolated exocyclic methylene group, one conjugated carbonyl unit in the α:β unsaturated lactone system and one hydroxyl group which loses easily a mole of water to lengthen the conjugation.

Nature of the remaining two hydroxyl groups was ascertained by recent workers (Cava & Weinstein, 1959) who observed that trityl chloride in pyridine converts andrographolide to a monotrityl derivative, $C_{30}H_{42}O_4$, m.p. 200° indicating thereby the presence of one primary hydroxyl group in the molecule. This trityl derivative is oxidised by chromium trioxide in pyridine to a ketoanhydrotritylether, acid hydrolysis of which gives off formaldehyde by way of retroaldol cleavage. Evidently andrographolide contains one secondary hydroxyl in β-position to the primary hydroxyl group.

On selenium dehydrogenation andrographolide produces a mixture of hydrocarbons which Karrer *et al.* (1951) called andrographene, $C_{14}H_{16}$, probably a mixture of tetra-alkylated napthalenes. The mixture gives a napthalene tetracarboxylic acid on treatment with alkaline potassium ferricyanide which forms a dianhydride. Andrographene forms a picrate, $C_{20}H_{19}O_7N_3$, m.p. 130-30.5° and a tetranitrobenzene compound, $C_{20}H_{19}O_6N_3$, m.p. 134°. The latter gives 1:2:5:6 tetramethyl napthalene on chromatography over alumina. Kondo & Ono (1951, 1953) also observed that slow dry distillation of andrographolide with selenium up to 300° for twenty-six hours produces neither a phenol nor an acid but gives a neutral oil showing fluorescence in ultraviolet light. It forms a mixture of picrates that on chromatography separates into five solids of which one is identified as 1:2:5:6 tetramethyl napthalene.

Diterpenoids are classified in dicyclic or tricyclic groups according to the nature of hydrocarbons they produce under selenium dehydrogenation. Thus while tricyclic diterpenes like abietic acid, d-pimaric acid and ferruginol produce phenanthrene derivatives, dicyclic diterpenes like sclareol, marrubin and agathic acid furnish napthalenes.

Isolation of 1:2:5:6 tetramethyl napthalene as a product of dehydrogenation will thus normally rank andrographolide in the family of dicyclic diterpenoids and Chakravarti *et al.* (1951) in fact suggested a similarity in the structure of andrographolide with that of marrubin (III), the bitter principle of *Marrubium vulgare* Linn., although they could not adduce any positive evidence to that end.



III

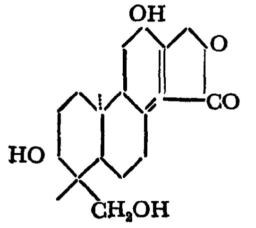
Andrographolide gives a fine red colour when treated with a strong methanolic solution of potassium hydroxide. Neither tetrahydroandrographolide nor desoxyandrographolide gives this colour. Isoandrographolide, m.p. 198-8.5° prepared by Karrer *et al.* (1951) by action of concentrated hydrochloric acid on andrographolide does not also produce this colour. Isoandrographolide lacks the α:β unsaturation but possesses a reducing centre; it contains however both the double bonds and so the possibility of any ring closure is eliminated. Kondo et al (1950) made an abortive attempt to cyclise andrographolide with formic acid and although Rangaswami & Rao (1951) failed to produce an isoandrographolic acid, it was reported by Moktadar & Guha Sirkar (1939).

Karrer *et al.* (1951) studied the oxidation of tetrahydroandrographolide and obtained a diketone, $C_{20}H_{30}O_5$, m.p. 192.5-4.5°. They also undertook the oxidation of diacetyldesoxyandrographolide with potassium permanganate to isolate an acid, which gave a methyl ester, $C_{19}H_{28}O_7$, m.p. 119.5-20°. It corresponds to a dihydromonoketo acid which these authors formulated as $C_{14}H_{22}O_5$. It does not impart any colour to ferric chloride solution and further the keto group, which they considered to be situated in a ring has not the carboxyl group in its β-position.

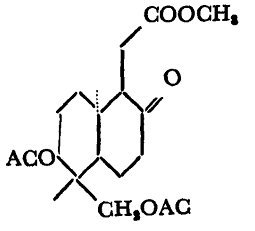
Kondo & Ono (1954) carried on the oxidation of triacetylandrographolide with potassium permanganate and obtained a small quantity of neutral crystals, m.p. 214-15° together with an acid, m.p. 172° giving a methyl ester, m.p. 75-8°. The oxidation of andrographolide was also studied by these workers very thoroughly but unfortunately no definite conclusion could be arrived at.

When such was the state of affairs from degradational evidences, physical data were taken into consideration to work out the fine structure of andrographolide. Uleipool & Kostermaus (1951) from a study of infra red spectrum showed that it contains a five-membered unsaturated lactone ring and a terminal methylene group. Cava and Weinstein (1959) extended the infra red observation to suggest structure IV for andrographolide.

They criticised the formula $C_{19}H_{28}O_7$ for the diacetoxy monoketo methyl ester as suggested by Karrer *et al.* and considered it to be $C_{20}H_{30}O_7$ having structure V. From a study of the optical rotatory dispersion of V, these authors concluded that andrographolide belongs to the class of compounds possessing wrong absolute configuration relative to the steroids. From a detailed analysis of the nuclear magnetic resonance of triacetyl andrographolide Chan & Haynes (1960) proposed structure VI for andrographolide, mainly to explain the presence of a proton attached to a doubly bonded carbon atom which is β and conjugated with a carbonyl group. Arya (1962) from a study of infra red and mass spectrum of andrographolide together with high energy electron bombardment on it, has shown that andrographolide contains an axial hydroxyl at C-3 in a bicyclic diterpene skeleton. He has further observed that the primary hydroxyl at C-4 is also axial in nature since andrographolide does not give any acetonoid compound.



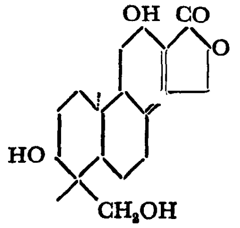
IV



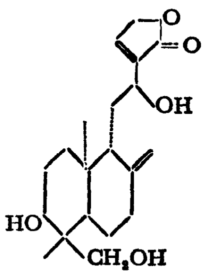
V

A closer examination of the two structures IV & VI, both suggested solely from physical evidences, will reveal that they differ in relative position of the C-12 hydroxyl with the carbonyl group of the lactone bridge. The secondary nature of the C-12 hydroxyl group has been assumed in both the structures without any proof. Both the structures fail to satisfactorily explain the formation of the desoxy diacetate which oxidises to compound V. This necessarily requires a rearrangement of the double bond. Lengthening of conjugation by dehydration is feasible in both the structure. But the possibility of an isoandrographolide is remote in structure VI, whereas it satisfactorily explains a hydrogen bonding between the carbonyl and C-12 hydroxyl as suggested by Chan (1960) for the observed frequency at 1725 cm^{-1}. The location of the exocyclic methylene group at C-8 is justified both from the product of dehydrogenation and also on biogenetic ground.

Cava *et al.* (1962) have very recently confirmed the structure VI and have put forward configuration VII for andrographolide which is mirror image of that found in the steroids and higher terpenoids.



VI



VII

Maiti, Kanji & Chatterjee (1959) while carrying out routine assay of various preparations of Kalmegh available in the market observed that both the methods of preparation of liquid extract of Kalmegh I.P. and its assay as recommended by the Indian Pharmacopoeia, 1955 were defective; a suitable modification for preparation of liquid extract of Kalmegh I.P. as well as a new method of assay of andrographolide were suggested.

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